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Adaptive Immunity against Streptococcus pyogenes in Adults Involves Increased IFN-γ and IgG3 Responses Compared with Children

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Each year, millions of people are infected with Streptococcus pyogenes, leading to an estimated 500,000 annual deaths worldwide. For unknown reasons, school-aged children have substantially higher infection rates than adults. The goal for this study was to provide, to our knowledge, the first detailed characterization of the human adaptive immune response against S. pyogenes in both children and adults. We report that all adults in our study, as well as most children, showed immunity against the two conserved group A streptococci (GAS) Ags, streptococcal C5a peptidase and immunogenic secreted protein. The response primarily consisted of three subsets of Th1 T cells, in which the TNF-α* and IL-2*TNF-α* subsets were most frequent. Humoral immunity was dominated by IgG1 and IgG3, whereas the Th2-associated IgG4 isotype was only detected at very low amounts. IgG3 levels correlated significantly with IFN-γ, but not with IL-5, IL-13, IL-17, or TNF-α. Interestingly, children showed a similar pattern of Ag-specific cytokine release, but displayed significantly lower levels of IgG3 and IFN-γ compared with adults. Thus, human immune responses against S. pyogenes consist of a robust Th1 cellular memory response in combination with IgG1/IgG3-dominated humoral immunity that increase with age. The significance of these data regarding both the increased GAS infection rate in children and the development of protective GAS vaccines is discussed. The Journal of Immunology, 2015, 195: 000–000.

Group A streptococci (GAS; Streptococcus pyogenes) are major human pathogens causing both suppurative and nonsuppurative infections. They give rise to a broad spectrum of clinical conditions ranging from uncomplicated infections, such as pharyngitis and impetigo, to life-threatening invasive diseases (1). Each year millions of people are infected, and it is estimated that GAS account for >500,000 deaths worldwide, mostly from invasive infections and complications following GAS infections such as rheumatic fever and rheumatic heart disease (2). Despite decades of research, there are no licensed vaccines against GAS, but vaccine candidates are currently under clinical (3–5) and preclinical investigation [reviewed in (6)].

The development/refinement of effective and safe vaccines against GAS is dependent on knowledge about the immune response needed to combat the infection. There is ample evidence that Abs develop after exposure to GAS bacteria (7–11) and that Abs have protective capacity (12–17). Consequently, vaccine development has focused onAbs and to a lesser extent on cellular T cell–based immunity. However, it is well known that T cell responses are essential for induction of high-affinity Abs, as well as for the longevity of the Ab-producing B cells, by providing survival and differentiation signals (18–20) and by inducing and maintaining memory B cells (21, 22). In addition, cytokines secreted from T cells, such as IFN-γ or IL-4, are responsible for guiding the class-switch recombination that results in particular Ig classes and subclasses (23–26). Th17 cells have been shown to protect against bacterial infections, such as Streptococcus pneumoniae (27, 28), and newer studies in GAS animal models have indicated that these cells may also play a role in protection against an infection with GAS (29–31). Taken together, several factors indicate that T cells, in addition to Abs, may play an important role in protective immunity against GAS.

In humans, very little is known about the cellular component of an anti-GAS immune response. A previous study indicated the presence of anti-GAS Th1 T cells, but, as whole bacteria were used in their stimulation assays, it cannot be excluded that the observed responses in part derived from innate cells (32). Regarding responses against single proteins, CD4 T cells (directed against GAS M protein) are found in tonsils of patients with recurrent tonsillitis and tonsillar hypertrophy, and these CD4 T cells proliferate and produce cytokines (IFN-γ, IL-2, IL-4, IL-5, and IL-6) (33). It has also been suggested that the M protein induces human regulatory T cells to suppress adaptive responses as a defense mechanism of GAS (34). Hence, it is clear that exposure to GAS bacteria in fact induces cellular immune responses in humans against the M protein, but the details of this response are still obscure. More importantly, nothing is known about the cellular immunity against protein targets other than the M protein. Because the M protein is highly variable, and immune responses to the M protein may be harmful to the host, studies with non-M protein Ags are important regarding the development of future GAS vaccines. Finally, as GAS pharyngitis is much less common in adults compared with school-aged children (35–39), a comparison of these age groups

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Abbreviations used in this article: GAS, group A streptococcus; IQR, interquartile range; Isp, immunogenic secreted protein; MFI, mean fluorescence intensity; ScpA, streptococcal C5a peptidase.

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we used the streptococcal C5a peptidase (ScpA) (40, 41) and immunogenic secreted protein (Isp) (42) to study the adaptive anti-GAS immune response in 59 healthy adults and children with no history of poststreptococcal sequelae. Our data show that anti-GAS immunity is mediated by specific subsets of CD4 T cells in combination with IgG3 and IgG1. We moreover demonstrate a correlation between IgG3 and IFN-γ and, interestingly, that the levels of IgG3 and IFN-γ are lower in children compared with adults.

Materials and Methods

Human subjects

We enrolled healthy adults >20 y and school-aged children from 5 to 15 y. Adults were included among staff at the Department of Infectious Disease Immunology at Statens Serum Institut. Children were included at the outpatient clinic at the Department of Pediatrics at Hvidovre Hospital among patients awaiting blood sampling for GAS-related conditions. Volunteers undergoing immunosuppressive treatment, reporting symptoms of acute or recent acute infections or other factors impacting immune responses, were excluded. The study was approved by the Committee on Health Research Ethics in the Capital Region (protocol H-2-2014-057) and the Danish Data Protection Agency (J.nr. 2014-54-0733).

Ags

Full-length coding sequences of ScpA (spy2010) and Isp (spy1801) were obtained from S. pyogenes M1 SRF730 and codon optimized for expression in E. coli. Amino acids Asp130, His193, and Ser512 were deleted in the sequence for ScpA to obstruct C5a peptidase activity, whereas aa 1–24 and aa 251–270 were deleted in Isp for ease of expression. The DNA sequences were synthesized (China Peptides) and mixed in DMSO at a concentration of 2 mg/ml as a positive control for each Ag. Peptides were volunteers with a confirmed GAS infection within the past 3 mo. Samples were added in duplicates in a 1:8 dilution, and IFN-γ was detected with 0.1 μg/ml biotin-conjugated mouse anti-human IFN-γ mAb (clone B1335; VWR–Bie & Berntsen) and 0.5 μg/ml HRP-conjugated streptavidin (Invitrogen Life Technologies). ELISA (E) was used as standard in a 2-fold dilution series going from 5000 to 19.5 pg/ml. The enzyme reaction was developed with 3,3’,5,5’-tetramethylbenzidine (TMB Plus; Kementec) and stopped with 0.2 M H2SO4. Plate readers at 450 nm with 620 nm correction were used to measure absorption at 492 nm with 690 nm correction.

Multiplex cytokine assay

The V-PLEX cytokine multiplex assay (Meso Scale Discovery) was performed according to the manufacturer’s instructions. In brief, 25-μl sample or standard was dispensed to each well in a 1:2 dilution. One standard curve was made for each plate. Plates were sealed with sealing tape and incubated at room temperature on a table shaker for 2 h. After washing three times with PBS plus 0.05% (v/v) Tween 20 (Merck), detection Ab was added to each well and plates were sealed and incubated as before. Plates were washed three times with PBS plus 0.05% (v/v) Tween 20, and 150 μl Read Buffer was added to each well before recording plates with a Sector Imager 2400 (Meso Scale Discovery). Calculation of cytokine concentrations in samples was performed by four-parameter logistic nonlinear regression analysis of the standard curve with MSD Discovery Workbench v4.0.12.

Measurements of Ab titers

Maxisorp microtiter plates (Nunc) were coated with ScpA or Isp (0.5 μg/ml), a tuberculosis control Ag (Rv3616c; 0.5 μg/ml), or tetanus toxoid (0.5 μg/ml). Plates were blocked with 5% skimmed milk (w/v) in PBS overnight at 4°C. After washing with 0.05% (v/v) Tween 20 in PBS, free binding sites were blocked with 3% skimmed milk (w/v) plus 0.05% (v/v) Tween 20 in PBS, followed by another washing step. Plates were incubated for 1 h with individual plasma samples in 10-fold serial dilutions in PBS containing 1% skimmed milk (w/v) plus 0.05% (v/v) Tween 20 in PBS, followed by another washing step. Plates were incubated for 1 h with individual plasma samples in 10-fold serial dilutions in PBS containing 1% skimmed milk (w/v) plus 0.05% (v/v) Tween 20 starting with a 1:10 dilution; further washing, plates were incubated for 1 h with HRP-conjugated secondary Abs (polyclonal rabbit anti-human IgG, Dako; mouse anti-human IgG, IgG2, IgG3, and IgG4, Life Technologies) diluted 1:6000 for IgG, 1:2000 for the four IgG subclasses in PBS containing 1% skimmed milk (w/v) plus 0.05% (v/v) Tween 20; Ag-specific Abs were detected by an enzyme reaction with 3,3’,5,5’-tetramethylbenzidine substrate. Reactions were stopped with 0.2 M H2SO4 after 30 min, and the OD was measured at 450 nm with 620 nm correction.

Data analysis and statistics

EC50 Values for Ab responses were calculated by a sigmoidal fitting of the data points in a 10-fold dilution series after a logaritmic transformation of the plasma dilution factor. Donors with EC50 <10 or with OD450nm values too low for accurate fitting had their EC50 adjusted to 10, which was the lowest dilution factor in the assay.

Results are either presented as individual data points for each donor or as box plots indicating the median and interquartile range (IQR) with whiskers indicating minimum and maximum. Statistical significance was evaluated with a Friedman test with Dunn’s multiple comparison posttest, when comparing immune responses against ScpA, Isp, and a control in the same set of donors (paired analysis). A Wilcoxon test was used to compare levels of cytokine-producing CD4 and CD8 T cells measured in the same donors (paired analysis). The Mann–Whitney U test was used to compare immune responses between children and adults as well as between IgG1/IgG3 high and low responders (unpaired analysis). A p value <0.05 was considered significant. Statistical analyses are also mentioned in the figure legends. Prism version 6.05 (GraphPad) was used for data visualization and statistical analysis.
Results

The humoral immune response against *S. pyogenes* is characterized by IgG1/IgG3 in adults

The purpose of this study was first to characterize the GAS-specific immune response in both healthy adults and children, and secondly to examine whether there were any significant differences in the anti-GAS immunity in these two age groups. Fifty-nine participants were included in the study from January 2014 to January 2015. None of the participants had symptoms of GAS infection at the time of enrollment and had no confirmed GAS infections within the past 3 mo. The participants were children between 5 and 15 y (n = 30) and adults of 24–63 y (n = 29). Gender distribution was 53% females, and the mean age ± SD was 11.2 ± 3.4 and 37.9 ± 10.5 y in the two age groups (median age 12 and 36, respectively). The children were included at the outpatient clinic at the Department of Pediatrics at Hvidovre Hospital among patients awaiting blood sampling for GAS-unrelated conditions. For this study, we chose the Ags ScpA (40, 41) and Isp (42). Both of these Ags are immunogenic and conserved among GAS strains. Thus, exposure to GAS (regardless of the strain) will most probably lead to an adaptive immune response against one or both of these Ags.

In our characterization of the humoral response, we found that levels of IgG against ScpA were higher than against Isp (median EC50 of 682, IQR 546–1297 versus median 185, IQR 110–272), but notably all participants showed elevated IgG EC50 against both GAS Ags compared with the background and to a *M. tuberculosis* control Ag (10, IQR 10–10) that was produced and purified in the same way as ScpA and Isp (Fig. 1A). To investigate the polarization of the Ab response, we next measured the plasma levels of Ag (ScpA)-specific IgG1–4 (Fig. 1B, 1D). The results showed that the most abundant IgG subclass was IgG3 with a median EC50 of 46.1 (IQR 12.7–313.9), followed by IgG1 (31.4, IQR 16.3–75.7) and IgG2 (11.2, IQR 10.0–31.1). In general, donors that were high in IgG2 also displayed IgG3 responses, and, in the majority of donors, IgG4 was found at low or undetectable levels. Although the responses were lower, a similar subclass profile was seen for Isp (analyzed in 14 donors; data not shown). As IgG3 is mostly associated with Th1 responses, and IgG4 is strictly Th2 associated (44–50), this suggested that the response induced by GAS is a Th1-polarized response. In comparison, the response induced by previous vaccinations with tetanus in aluminum hydroxide (used in this study as a Th2 control) was, as expected, dominated by IgG1 and IgG4 (Fig. 1C, 1E) with EC50 medians of 39.7 (IQR 17.5–76.3) and 43.7 (IQR 10–383.3), respectively. In summary, all participants showed a significant anti-GAS IgG response against ScpA and Isp, which was dominated by IgG1 and IgG3. In contrast, IgG4 responses were low or undetectable.

The cellular immune response against GAS is Th1 polarized

Cellular immunity in humans against non-M protein GAS Ags has not previously been described. To examine this, PBMCs from fresh heparinized blood from healthy adults were incubated with or without ScpA or Isp. Based on a small pilot experiment in which culture supernatants were harvested at different time points (Supplemental Fig. 2A), we first examined the Th1 cytokine IFN-γ in culture supernatants after 7 d of stimulation (Fig. 2A). IFN-γ release >100 pg/ml was used post hoc as an arbitrary cutoff to differentiate responders from nonresponders. Eighty-six percent of the donors responded to ScpA with a cellular IFN-γ response, and 69% responded to Isp, whereas 90% responded to either of the two Ags.
Responders showed substantial differences in the levels of IFN-γ ranging from 100 to 22,715 pg/ml with a median of 2,907 pg/ml (IQR 425–7,040) for ScpA and 587 pg/ml (IQR 19–1,998) for Isp. The same responder frequency, and a similar median IFN-γ response, was obtained by stimulating with a pool of overlapping peptides for Isp (Supplemental Fig. 2B).

Having established that most adults harbor a GAS-specific cellular immune response, we decided to expand these analyses to cytokines other than IFN-γ by using a multiplex panel of Th1, Th2, and Th17 cytokines. Stimulation with ScpA and Isp prompted a significant release of all of the tested cytokines compared with the media control (Supplemental Fig. 2C). However, only lower levels were detected for IL-5 and IL-13 with median responses of 11 and 44 pg/ml, respectively, for ScpA and 10 and 12 pg/ml for Isp (Fig. 2B).

Overall, the highest levels of cytokines were found for IFN-γ, followed by TNF-α and IL-17 with medians of 2907, 205, and 120 pg/ml for ScpA and 587, 104, and 56 pg/ml for Isp.

These results demonstrated that cellular GAS immunity in humans involves cytokines associated with Th1 and Th17 cells (and only to a minor extent Th2 cells) and that GAS-specific cellular immune responses against conserved Ags are common in the adult population.

**CD4 T cells belonging to three specific subsets dominate T cell–induced anti-GAS immunity**

We next examined which subsets of T cells were responsible for the cellular immune response. Ag-specific CD4 and CD8 T cells expressing any combination of IFN-γ, TNF-α, IL-2, and IL-17 were examined by flow cytometry using intracellular cytokine staining (for gating strategy, see Supplemental Fig. 1).

**High IgG3 responses correlate with higher IFN-γ levels**

Cytokines secreted by T cells are known to influence Ab responses (23–26). The fact that we found a Th1 polarization of both the humoral and cellular response prompted us to investigate whether there was a correlation between Ab levels and the cytokine response. For the two most dominant IgG subclones, IgG1 and IgG3, we divided the adult donors into high and low responders based on whether their EC50 values were above or below the median, respectively (Fig. 4A, 4E). IgG1/IgG3 high and low responding donors were then compared for their expression of IFN-γ, TNF-α, and IL-17 that were found as the most dominant cytokines (Fig. 2B). IgG1 low donors had a trend for higher IL-17 levels than IgG1 high responding donors (median 319, IQR 68–554 compared with 54, IQR 23–177, p = 0.102), whereas levels of IFN-γ and TNF-α were similar (Fig. 4B–D). In contrast, IgG3 high responders had significantly higher IFN-γ levels than IgG3 low responders (median 6,484, IQR 1,507–12,831 compared with 1,461, IQR 172–3,345, p = 0.0349), whereas TNF-α and IL-17 were expressed at similar levels (Fig. 4F–H). As expected, we hereby confirm that the Th1 polarization of the IgG response (IgG3 > IgG4) was associated with the strong cellular IFN-γ response.

**Adults express higher levels of IFN-γ and IgG3 compared with children**

In school-aged children, the rate of GAS pharyngitis is generally higher than in adults (35–39). To examine whether this correlates with qualitative or quantitative differences in the anti-GAS immunity compared with adults, we enrolled 30 children from 5 to 15 y in a new study.

Like with adult donors, we first characterized the cytokine secretion in cultures of PBMCs stimulated with ScpA and Isp.
Children had a combined cellular responder frequency against both ScpA and Isp (donors with IFN-γ levels >100 pg/ml) of 89% (compared with 90% in adults). The cytokine pattern was also similar to that observed in adults, and thus dominated by IFN-γ, TNF-α, and IL-17 (Supplemental Fig. 3A). Moreover, the cellular response in children involved CD4 T cells that belonged to the same three subpopulations observed in adults, namely TNF-α+–, TNF-α+IL-2+–, and TNF-α+IL-2+IFN-γ+–expressing cells (Supplemental Fig. 3B–F). However, a closer comparison between adults and children revealed differences in the magnitude of the responses. IFN-γ levels were significantly lower in children compared with adults (median 678, IQR 36–3229 compared with 2907, IQR 425–7040 pg/ml, \( p = 0.0450 \)), in contrast to all the other cytokines analyzed (Fig. 5A–E). This correlated with a reduction in IgG3 expression (from a median EC50 of 24, IQR 10–55 in children to 46, IQR 11–308 in adults, \( p = 0.0586 \)), whereas the other IgG isotypes were found at similar levels (Fig. 5F–J). In particular, for IgG3 there was a trend that the IgG3 response increased with age, that is, IgG3 levels in adolescents were higher than in young children, but lower than in adults. Consequently, when comparing adults with the young children (5–12 y), the difference in IgG3 was even more obvious (\( p = 0.0215 \)) (Fig. 5K), which was not the case for the other subclasses (data not shown).

The raw data for the IgG subtyping in the different age groups are as shown in Supplemental Fig. 4.

In summary, although adults and children show the same overall type of immunity (Th1/IgG1/IgG3), we observed increased levels of both IgG3 and IFN-γ in adults.

Discussion
This study characterizes the immune response against non-M protein Ags in children and adults induced by infection with \( S. \) pyogenes.

All of the adult donors that were included in this study had elevated levels of ScpA- and Isp-specific IgG compared with a recombinant control Ag. Except for two donors, this was also observed for children. This suggests that immune responses against GAS develop in all humans over time. In addition to Ab responses, we showed that GAS exposure led to formation of Ag-specific cellular responses and that these cellular responses are very common, because as much as 90% of the adults and 89% of the children had an IFN-γ response toward either ScpA or Isp. It was striking that we measured IFN-γ responses as high as 22,715 pg/ml and median levels of 2,907 pg/ml IFN-γ for ScpA and 587 pg/ml for Isp. In comparison, PBMCs from patients with \( M. \) tuberculosis infections release 1,100–1,200 pg/ml IFN-γ on
average after stimulation with two immune-dominant tuberculosis Ags in a similar stimulation assay (52). Although IFN-γ levels would be expected to vary under different laboratory settings and culture conditions, this comparison nonetheless indicates that the GAS cellular immune responses determined in this study are relatively strong. Multiplex cytokine analysis confirmed that the cellular responses against GAS were Th1 dominated with high levels of TNF-α and IFN-γ compared with the other cytokines that were examined (IL-5, IL-13, and IL-17). This is in agreement with published studies showing that GAS bacteria stimulates human macrophages and dendritic cells to secrete large amounts of Th1-inducing cytokines (53–56). In both adults and children, the most frequent T cell subsets determined by flow cytometry were TNF-α⁺, TNF-α⁺IL-2⁺, and TNF-α⁺IL-2⁺IFN-γ⁺, and, in agreement with previous observations (57, 58), we observed the highest expression of TNF-α and IL-2 in TNF-α⁺IL-2⁺ and TNF-α⁺IL-2⁺IFN-γ⁺ cells. In particular, the TNF-α⁺, TNF-α⁺IL-2⁺ subsets are associated with memory subsets (51), indicating that the relatively high exposure rate that is expected for a common bacterium like GAS (especially in children) does not prevent formation of GAS-specific central memory T cells in both adults and children.

**FIGURE 4.** High IgG3 responses correlate with increased IFN-γ levels. For both IgG1 and IgG3, adult donors were grouped into high responders (donors with EC₅₀ higher than the median, n = 15) and low responders (donors with EC₅₀ lower than the median, n = 14). (A and E) These two donor subsets were then compared for their expression of IFN-γ, TNF-α, and IL-17 (the three most dominant cytokines) for both IgG1 (B–D) and IgG3 (F–H). Boxes span the IQR around the median, and whiskers indicate minimum and maximum. The p values were calculated with a Mann–Whitney U test. These data points are all a subset of the ones shown in Figs. 1 and 2.

**FIGURE 5.** Adults display higher levels of IFN-γ and IgG3 than children. Comparison of the immune response in adults >20 y (n = 29) and children aged 5–15 y (n = 30) based on ScpA-binding IgG subclasses (A–E) and cytokines released by PBMCs stimulated by ScpA (F–J), measured as described in Figs. 1 and 2 (for the adults). For two children only plasma IgG was analyzed. Boxes span the IQR around the median, and whiskers indicate minimum and maximum. The p values were calculated with a Mann–Whitney U test. (K) IgG3 is shown for young children (5–12 y), old children (13–15 y), and adults (>20 y). The p value was calculated with a Mann–Whitney U test between young children and adults.
Our finding of GAS-specific Th17 T cells in circulation of both adults and children is interesting, as there have been indications in experimental animal models that Th17 cells are involved in the protection against mucosal infection with GAS (29–31) or with S. pneumoniae (27, 28). However, Th17 cells are mostly found in peripheral tissues and organs (59–63), and, because our characterization of the immune responses was limited to the blood, human Th17 responses might be even more pronounced at mucosal sites.

One central question is what role the Th1 cells play in GAS immunity in humans, and one role may be to influence the Ab response. Abs in human immune serum most probably kill bacteria via neutrophils, and this mechanism is dependent on complement receptors, demonstrating the importance of complement deposition in acquired immunity to GAS (64). The IgG subclasses vary in their ability to bind C1q and activate the complement cascade via the classical pathway (IgG3 > IgG1 > IgG2 > IgG4) (65, 66), and the Ab responses we report for ScpA were dominated by IgG1 and IgG3, whereas IgG4 was only found in low or undetectable levels. This is consistent with another study reporting that the most abundant subclasses against the M protein were IgG1 and IgG3 (67). IgG1 is associated with neither Th1 nor Th2 responses, whereas IgG3 is mostly associated with Th1 responses (44–50), indicating that the Th1 response that we identified may be involved in regulating the Ab response against GAS toward IgG3. In support of this, we found that donors with high IgG3 levels also had significantly higher IFN-γ responses, altogether suggesting that one effector function of the Th1 cells is to guide the IgG response into production of the most complement-fixing IgG subclass (and away from the IgG4 that does not fix complement). Our observation that children had lower levels of IgG3 and IFN-γ (but not of the other IgG isotypes and cytokines analyzed) compared with adults is of particular interest in this context. Whether these differences are substantial enough to make a biological impact is presently not known, but it could be speculated that the increased Th1/IgG3 immunity in adults might play a role regarding the reduced GAS infection rate compared with children. This observed difference in Th1/IgG3 immunity might reflect a reduced ability of children to respond to infections with S. pyogenes and/or that adults simply have had more encounters with the bacterium and therefore develop stronger immune responses.

Apart from influencing the IgG isotype response, IFN-γ (secreted by T cells and/or innate cells) may also exert more direct effector functions, for example, by activating macrophages. It has been suggested that IFN-γ can prevent local dissemination of GAS bacteria (68) and is indispensable for protection against a lethal skin infection in mice (69). Thus, considering the potential effect of IFN-γ on innate cell activation in combination with its IgG3-inducing capabilities, it can be suggested that future vaccines against GAS should aim at inducing Th1 responses. Adjuvants such as aluminum hydroxide, that is, inducing Th2/IgG4 responses, may therefore constitute a suboptimal adjuvant for a vaccine against GAS. In fact, we recently learned that ScpA formulated with aluminum hydroxide was significantly less protective than ScpA given in a Th1-inducing adjuvant such as CAF01 (J. Dietrich, unpublished observations). Moreover, this is in agreement with published results involving a S. pneumoniae vaccine (70).

In conclusion, our results demonstrate that GAS adaptive immune responses are very frequent and involve both strong Ab and cellular immune responses. The identified cellular Th1/Th17 memory responses in children and adults are likely to play an important role in the development and/or maintenance of GAS immunity. We suggest that at least one role is to facilitate induction of complement-fixing IgG3 Abs. Importantly, children showed lower levels of both IFN-γ and IgG3, which may play a role regarding the higher rate of GAS infections in children, something that will be a subject for future studies.

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**Disclosures**

The authors have no financial conflicts of interest.

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